

Estimating Ruminant Protein Degradability of Roasted Soybeans Using Near Infrared Reflectance Spectroscopy^{1,2}

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ABSTRACT

Heat treatment by roasting is used extensively in the Midwest to increase RUP of whole soybeans (*Glycine max* L. Merr.). Samples of roasted soybeans (n = 266) were collected from different suppliers in Wisconsin, Minnesota, and Michigan to determine the value of near infrared reflectance spectroscopy for estimating RUP. Samples were analyzed for DM, total N, protein dispersibility index, and RUP using an inhibitor in vitro procedure. Samples were milled (1 mm), and near infrared reflectance spectra were collected. Scans from 121 samples (calibration set) were used to develop calibration equations. Standard errors and coefficients of multiple determination for calibration of near infrared reflectance spectroscopy using conventional chemical assays were, respectively, 0.26 and 0.97 (DM), 0.05 and 0.99 (total N), 1.60 and 0.71 (protein dispersibility index), and 0.98 and 0.90 (RUP). A validation set of 145 samples then was used to evaluate the accuracy of calibration equations for estimating chemical composition. Standard errors and coefficients of determination for validation were, respectively, 0.63 and 0.86 (DM), 0.12 and 0.86 (total N), 3.52 and 0.52 (protein dispersibility index), and 1.54 and 0.70 (RUP). Protein dispersibility index was poorly correlated ($r^2 = 0.28$) to RUP estimated by inhibitor in vitro. Results indicated that near infrared reflectance spectroscopy might be used to estimate DM, total N, and RUP in roasted soybeans.

(**Key words:** roasted soybeans, protein degradability, near infrared reflectance spectroscopy)

Abbreviation key: IIV = inhibitor in vitro, NIRS = near infrared reflectance spectroscopy, PDI = protein dispersibility index, RSB = roasted soybeans, RUPCR = RUP corrected by regression.

INTRODUCTION

The absorbable protein requirement of high producing dairy cows in early lactation cannot be met solely by ruminal microbial synthesis. The economic value of supplemental protein fed to dairy cows is determined largely by the amount that escapes ruminal degradation and is available for digestion and absorption in the small intestine. Roasting is a new treatment being used extensively in North America to increase RUP of whole soybeans (*Glycine max* L. Merr.). The Maillard reaction is the major process by which heating protects protein from ruminal degradation. In its early stages, the Maillard reaction causes only small losses in nutritionally available Lys, the AA that is most sensitive to heat damage (2), but substantially reduces ruminal protein degradation (14). This characteristic can be used to reduce microbial degradation without a major loss of intestinally released AA in properly heated soybeans (13). The effect of heat treatment is a function of both temperature and time of heat exposure. Faldet et al. (14) suggested that the optimal temperature of soybeans leaving the roaster ranges from 140 to 160°C and that those temperatures should be maintained for 120 to 130 min, respectively, to produce a roasted soybean (RSB) supplement with about 60% RUP.

The dairy industry needs a rapid, inexpensive, and accurate method to identify optimally heated protein supplements for lactating cows. An inhibitor in vitro (IIV) system was developed by Broderick (7) to estimate rate and extent of protein degradation in the rumen. The IIV procedure successfully predicted relative differences in lactation performance of cows fed solvent and expeller soybean meal (6, 8) and identified the optimal extent of heating required for protecting protein in soybeans (14, 15). The protein dispersibility index (PDI) has the potential to identify optimally heated soybeans (17); however, both

Received November 28, 1994.

Accepted October 6, 1995.

¹Mention of a trademark or proprietary product in this paper does not constitute a guarantee or warranty of the product by the Agricultural Research Service, the USDA, or Agriculture and Agri-Food Canada and does not imply its approval to the exclusion of other products that also may be suitable.

²Journal paper number 516 of the Agriculture and Agri-Food Canada Soils and Crops Research and Development, Sainte-Foy, QC, Canada.

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methods are too costly and time consuming for routine testing of commercial RSB. Methods based on near infrared reflectance spectroscopy (NIRS) are now used widely to estimate chemical composition of forages and other commercial livestock feeds. The purpose of this study was to determine whether NIRS could be used as a rapid, inexpensive, and accurate method for testing the protein in RSB for ruminal escape.

MATERIALS AND METHODS

Samples ($n = 273$) of RSB were collected between August 1991 and December 1992 from about 150 farmers and other commercial sources in Wisconsin, Minnesota, and Michigan. Samples were milled (1-mm screen, Wiley Mill; Arthur H. Thomas Co., Philadelphia, PA) and analyzed for DM by drying at 105°C for 48 h for total N by the Dumas method (4) and for PDI as described by Hsu and Satter (17). This procedure was a modification of the PDI method described by Eichner and Wolf (12) and the American Oil Chemists Society (3). Solubility of CP in various solvents has been used widely to estimate ruminal protein degradation of feedstuffs. This use stems from greater degradation of readily solubilized compounds than less soluble counterparts. The PDI method is based on solubility in distilled water. Protein solubility can be a simple and useful technique to measure treatment effects within a protein source but may lead to serious error when applied across diverse groups of feeds (27). On average, the PDI method was satisfactory for identifying RSB with well-protected protein; however, the substantial changes in RUP that attend only small differences in PDI reduce the predictive value of this method.

The most promising *in vitro* systems involve incubation of protein with mixed ruminal microorganisms. An IIV method using strained ruminal fluid enriched with particulate organisms has been developed by Broderick (7). This method assesses degradation from accumulation of protein breakdown products (NH_3 and total AA). Quantitative recovery of NH_3 and AA is achieved by incorporating hydrazine sulfate and chloramphenicol, inhibitors of microbial N metabolism, into the inoculum. This method appears to be more sensitive to differences in rate and extent of protein degradation for feedstuffs than CP solubility and ficin protease methods (7) and the *in situ* method (15). Therefore, rates of ruminal protein degradation for RSB were determined with the IIV procedure (7) scaled down to use 5 ml of McDougall's buffer (22) plus 10 ml of inoculum (total volume = 15 ml). Microbial activity was stopped by addition of

TCA to a final concentration of 5% (wt/vol). Net release of NH_3 and total AA between 0 and 4 h of incubation was used to estimate fractional degradation rates, assuming that protein degradation was first order. The RUP was estimated using IIV degradation rates and assuming a fractional passage rate from the rumen of 0.06/h. The RUP values were corrected by regression (RUPCR) for the escapes obtained for three reference proteins (casein, solvent soybean meal, and expeller solvent soybean meal) included in each incubation run. Values of RUPCR were expressed as percentages of DM: $\text{RUPCR} = [\text{RUPCR} (\% \text{ of CP}) \times \text{CP} (\% \text{ of DM})]/100$.

The 273 RSB samples then were ground with a cyclone mill (Udy Corp., Fort Collins, CO) fitted with a 1-mm screen, and the NIRS spectra were obtained (NIRSystem 6500 spectrophotometer; Perstorp Analytical, Silver Spring, MD). Samples were packed into a cylindrical sample holder designed by the manufacturer for powdered materials and equipped with a quartz window and scanned as described by Marten et al. (21). Seven of the samples were identified from NIRS spectra as outliers (21) and discarded; only data from the remaining 266 samples were used in this study. Scans from 121 samples (calibration set) were used to develop calibration equations for predicting DM, total N, PDI, and RUPCR. The calibration was made by using the modified, partial least squares regression method with Infrasoft International® software, version 2.0 (30). This method was a modified form of principal component regression and used all wavelengths identified in the segment to develop the equation. The modification involved standardizing the variables after each iteration. Cross-validation, to minimize overfitting of the equation, was conducted with predicted values. Equations selected from calibration statistics were used to compute DM, total N, PDI, and RUPCR of another set of RSB samples. This validation set, consisting of 145 samples, was then used to evaluate the accuracy of calibration equations. Data from chemical analyses were compared with data predicted by NIRS.

RESULTS AND DISCUSSION

Mean total N content (percentage of DM) for the 266 RSB samples was 6.73% (SE = 0.02) and ranged from 5.29 to 9.04% (Table 1). Mean PDI (percentage of CP) for the 266 RSB samples was 13.46 (SE = 0.30). Standardized PDI accepted in the industry for RSB are as follows [(28); L. D. Satter, 1994, personal communication]: PDI in the range from 9 to 12% correspond to optimal RUP of RSB, PDI in the range from 12 to 14 indicate that RUP of RSB may be

TABLE 1. Dry matter, total N, protein dispersibility index (PDI), and RUP corrected by regression (RUPCR)¹ concentrations for the total set of roasted soybeans samples (n = 266)² used in near infrared spectroscopic analysis.

Analysis	Mean	Median	Minimum	Maximum	SE
DM, %	94.49	94.57	86.04	98.29	0.11
N, % of DM	6.73	6.74	5.29	9.04	0.02
PDI, % of total CP	13.46	12.53	5.48	61.85	0.30
RUPCR, % of total CP	56.64	56.76	32.75	76.10	0.39
RUPCR, ² % of DM	23.80	23.69	13.79	32.22	0.18

¹Values obtained for reference proteins and expressed as a percentage of DM.

²Excluding 7 samples, which were identified as outliers based on near infrared spectra (21), from the 273 samples that were analyzed originally.

marginal, and PDI >14 indicate the RSB have been underheated. Based on these criteria, 32% of RSB samples (n = 85) were underheated (PDI ≥14), 28% (n = 75) were marginally heated (PDI = 12 to 13.99), and 35% (n = 92) were heated optimally for RUP (PDI = 9 to 11.99). About 5% (n = 14) had PDI <9 and might have been overheated.

The RUP content of RSB varies greatly (13), ranging from being similar to raw soybeans (about 25% RUP, % of CP) to being nearly equal to high cost, resistant proteins such as fish meal (about 65% RUP). Occasionally, overheating (>70% CP escape) may substantially reduce protein value, but soybeans roasted on the farm and by commercial processors most often are underheated. A realistic target would be 60% RUP [(28); L. D. Satter, 1994, personal communication], and about 70% of the RSB samples analyzed in this study had an RUPCR value (percentage

of CP) <60%. Mean RUPCR (percentage of CP) over all samples (n = 266), as estimated by IIV, was 56.64% (SE = 0.39), with a range of 33 to 76% (Table 1; Figure 1). Only 2 samples (0.8%) were between 30 and 39% estimated RUP, 21 samples (7.9%) were between 40 and 49%, 164 samples (61.7%) were between 50 and 59%, 66 samples (24.8%) were between 60 and 69%, and only 13 samples (4.9%) were between 70 and 79% estimated RUP.

The PDI values were not closely correlated with RUPCR values, regardless of whether RUPCR was expressed on a CP or DM basis (Figure 1), possibly because PDI is a measure of protein solubility but the IIV method estimates microbial protein degradation. Mahadevan et al. (18) and Nugent et al. (25) suggested that structural characteristics of protein (e.g., number of disulfide bonds), rather than solubility, have greater influence on rates of protein degrada-

TABLE 2. Calibration and validation statistics for near infrared reflectance spectroscopic (NIRS) analysis of roasted soybeans.¹

Item	Laboratory method		NIRS Method				R ²	r ²
	\bar{X}	SE	\bar{X}	SE	PLS ²	Transformation ³		
Calibration (n = 121)								
DM, %	94.64	1.10	94.61	0.26	9	2, 4, 4, 1	0.97	...
N, % of DM ⁴	6.70	...	6.71	0.05	7	1, 10, 10, 1	0.99	...
PDI, % of total CP ⁴	13.53	...	12.83	1.60	9	1, 4, 4, 1	0.71	...
RUPCR, % of total CP	56.10	3.09	56.60	2.41	7	2, 4, 4, 1	0.88	...
RUPCR, % of DM	23.49	1.28	23.64	0.98	6	2, 4, 4, 1	0.90	...
Validation (n = 145)								
DM, %	94.51	1.03	94.64	0.63	0.86
N, % of DM ⁴	6.75	...	6.73	0.12	0.86
PDI, % of total CP ^{4,5}	13.41	...	12.57	3.52	0.52
RUPCR, % of total CP ⁵	56.72	2.41	57.10	3.84	0.59
RUPCR, % of DM	23.93	1.11	24.04	1.54	0.70

¹PDI = Protein dispersibility index, R² = coefficient of multiple determination from calibration, r² = coefficient of determination from validation, and RUPCR = RUP corrected by regression, using three reference proteins.

²Number of terms in the equation calibrated using the modified partial least squares (PLS) regression method.

³Order of derivative function, segment length (nanometers), segment length (nanometers) of first smoothing, and segment length (nanometers) of second smoothing (1).

⁴Only single determinations were used in the laboratory analyses of N and PDI.

⁵The NIRS calibrations not conducted on PDI as a percentage of DM.

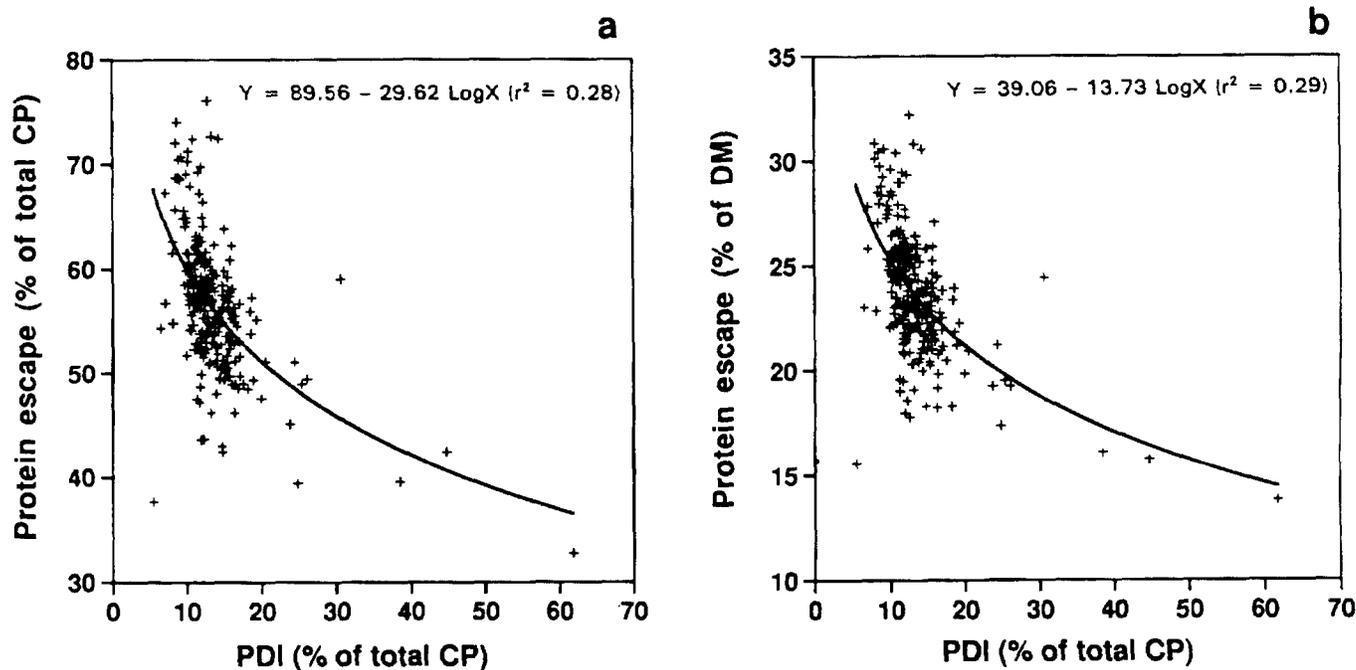


Figure 1. Relationships between protein dispersibility index (PDI; X) and a) RUP corrected by regression (RUPCR), expressed as a percentage of total CP, or b) RUPCR, expressed as a percentage of DM (Y). Determined on 266 samples of roasted soybeans.

tion. Protein solubility is confounded by the proportions of CP present as NPN and indigestible N fractions (7).

Because NIRS scanning was conducted on dry, ground, and unextracted samples, RUPCR was recalculated as a percentage of DM to determine whether the NIRS prediction would be improved. Mean RUPCR (percentage of DM) was 23.8% (SE = 0.18). The NIRS prediction was effectively better for the RUPCR expressed as a percentage of DM instead of as a percentage of CP (Table 2). Coefficients of multiple determination (R^2) obtained from NIRS calibration for DM, total N, PDI, and RUPCR (percentage of DM) were 0.97, 0.99, 0.71, and 0.90, respectively (Table 2). Validation data from the predictions (Table 2) are shown in Figure 2. Coefficients of determination (r^2) for estimating chemical composition by NIRS ranged from 0.52 for PDI to 0.86 for DM and total N; r^2 for RUPCR (percentage of DM) was intermediate (0.70). The standard error of validation (prediction) for NIRS was greater than the standard error of calibration, especially for PDI (Table 2; Figure 2). Better estimations with NIRS were obtained for IIV than for PDI. Thus, estimation of RUP directly by NIRS should be more accurate than estimation of PDI by NIRS and prediction of RUP indirectly from the estimated PDI.

The use of NIRS to estimate ruminal protein degradation characteristics of RSB seemed logical because NIRS has been used to estimate other bioassays, such as in vitro DM digestibility. Norris et al. (24) first showed the feasibility of using NIRS as a rapid technique for estimating in vitro DM digestibility and digestible DM in hays. Similar results were obtained later with grass silages (5). Eckman et al. (11) reported that NIRS was more precise than chemical determinations for estimating intake and digestible energy in forages. Givens et al. (16) demonstrated that the use of NIRS to predict in vivo OM digestibility in straws was more accurate than the use of ruminal fluid or cellulase-based procedures. Marten et al. (19) reported the R^2 of calibration for in vitro DM digestibility by NIRS was 0.92; validation data showed that the r^2 between predicted and chemical analyses were between 0.79 and 0.88, depending on locations and years. Marten et al. (20) reported that R^2 for NIRS calibrations for in vitro DM digestibility ranged from 0.82 to 0.97 for five different groups of forages. Bughrara et al. (9) reported that the best equation for true in vitro digestibility in alfalfa herbage had R^2 of 0.93 for calibration ($n = 52$) and had r^2 of 0.90 for validation ($n = 26$). Clark and Lamb (10) surveyed data from 16 different studies

using NIRS to measure forage in vivo digestible DM and in vitro DM digestibility and found that standard errors and R^2 varied, respectively, from 1.17 to 3.59% and 0.78 to 0.97 during calibration and standard errors and r^2 varied, respectively, from 1.16 to 4.40% and 0.66 to 0.95 during validation.

A discriminant analysis (26) was conducted on the validation sample set to compare the reliability of the RUPCR analysis by NIRS and the PDI procedure as methods for identifying RSB that were, as determined in the IIV assay: underheated (<20% RUPCR, % of

DM), marginally heated (20 to 24% RUPCR), optimally heated (24 to 28% RUPCR), or overheated (>28% RUPCR). Both the NIRS and PDI methods correctly identified five of nine underheated samples of RSB (Table 3). However, the NIRS procedure was more precise than the PDI in classifying RSB for RUP, correctly identifying 75, 73, and 70% of the samples that were marginally heated, optimally heated, and overheated, respectively (Table 3). The NIRS method correctly placed 125 out of 126 samples in either the marginally heated or optimally heated

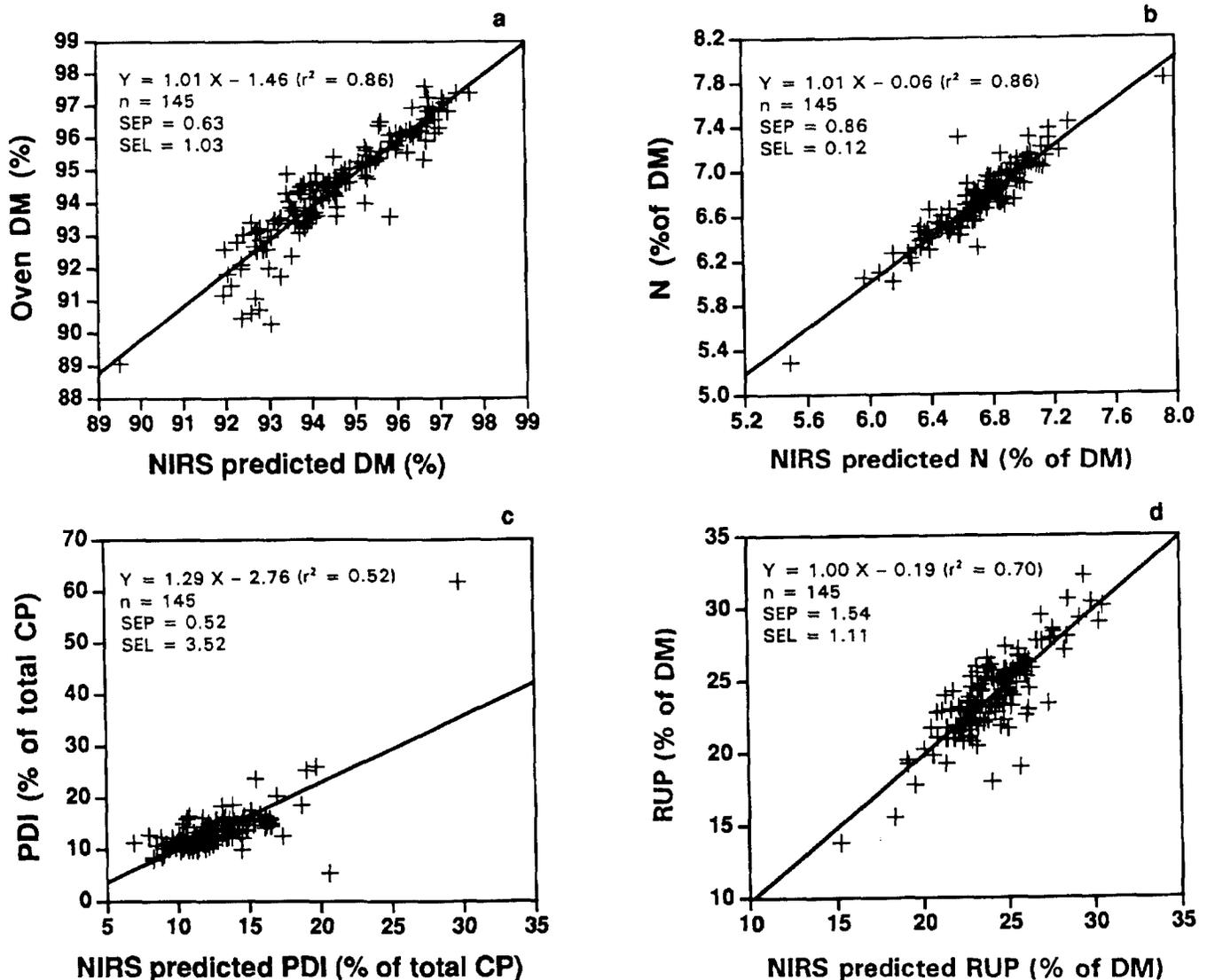


Figure 2. Relationships among a) DM, b) total N, c) PDI, and d) RUP of roasted soybeans, predicted by near infrared reflectance spectroscopy (X) and measured by chemical or in vitro assay (Y). SEL = Standard error of the laboratory; SEP = standard error of performance of the equation.

TABLE 3. Discriminant analysis on the validation set of 145 roasted soybeans comparing the number classified by RUP corrected by regression (RUPCR), determined using the standard inhibitor in vitro (IIV) method, with the number classified by the near infrared reflectance spectroscopic (NIRS) and protein dispersibility index (PDI) methods.

IIV Class ¹ and RUPCR	Classified by IIV (no.) (%)		Number classified by NIRS ²				Number classified by PDI ³			
			A (<20)	B (20-23.99)	C (24-27.99)	D (>28)	A (>14)	B (12-13.99)	C (9-11.99)	D (<9)
A <20, % of DM	9	6.21	<u>5</u> ⁴	3	1	0	<u>5</u>	1	2	1
B 20-23.99, % of DM	67	46.21	0	<u>50</u>	17	0	30	<u>21</u>	16	0
C 24-27.99, % of DM	59	40.69	0	15	<u>43</u>	1	7	20	<u>31</u>	1
D >28, % of DM	10	6.90	0	0	3	<u>7</u>	1	1	6	<u>2</u>
Totals no.	145		5	68	64	8	43	43	55	4
%	100.00		3.45	46.90	44.14	5.52	29.66	29.66	37.93	2.76
Classified by IIV no.			9	67	59	10	9	67	59	10
%			6.21	46.21	40.69	6.90	6.21	46.21	40.69	6.90

¹Classes correspond to those described by Satter et al. (28) and L. D. Satter, 1994, personal communication: A) underheated (RUPCR <49% of total CP), B) marginally heated (50 to 59.9% RUPCR), C) optimally heated (60 to 69.9% RUPCR), and D) overheated (>70% RUPCR).

²Percentage of RUPCR as a percentage of DM.

³As a percentage of total N.

⁴Values underlined in each row represent the number of roasted soybeans samples that were classified correctly by the NIRS and PDI methods; other values in each row are the number of samples that were classified incorrectly.

categories; samples were also identified by the IIV assay as being marginally or optimally heated. The PDI method tended to inflate the number of RSB that were identified as underheated; 45, 34, and 60% of those that were marginally heated, optimally heated, and overheated, respectively, were misassigned to the next lower RUP category (Table 3).

The NIRS results appeared to fit protein degradability data as well as data from other bioassays such as the in vitro DM digestibilities previously cited (10, 16, 19, 20). The NIRS technology has been widely exploited for predicting the nutritive value of forages and other livestock feeds. Although NIRS is an empirical technique, many convincing reports indicate that NIRS will accurately predict results from bioassays. Furthermore, NIRS estimates are at least as accurate as Kjeldahl determinations of N concentration of soybeans (23, 29) and as accurate as oven-drying for DM determination.

CONCLUSIONS

The results of the present experiments indicated that NIRS may be used to predict DM and total N in RSB. Standard errors of prediction from the NIRS

validation and coefficients of determination between NIRS estimates and chemical analyses were, respectively, 0.63 and 0.86 (DM) and 0.12 and 0.86 (total N). As a chemical assay, PDI was poorly correlated ($r^2 = 0.28$) to RUP determined by IIV and poorly estimated by NIRS (SEP = 3.52 and $r^2 = 0.52$). Estimating RUP by NIRS was more precise than the PDI technique for classifying RSB that were marginally or optimally heated. Standard errors of prediction and coefficient of determination were intermediate for RUP (RUPCR) as determined by the IIV procedure and expressed as a percentage of DM: 1.54 and 0.70, respectively. Further research is needed to confirm that the NIRS method can be used successfully to estimate RUP in RSB and other feedstuffs.

ACKNOWLEDGMENTS

The authors gratefully thank D. B. Ricker and M. C. Becker for their excellent technical assistance and D. Taysom of Dairyland Laboratories, Inc. (Arcadia, WI) for his assistance in obtaining samples of RSB.

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